reactivity in man exceeding that produced by the Reference Smallpox Vaccine.

[38 FR 32068, Nov. 20, 1973, as amended at 41 FR 51010, Nov. 19, 1976; 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 630.71 Production.

Vaccinia virus used for the manufacture of vaccine shall be obtained from vesicles on the skin of an inoculated calf or from inoculated chorioallantoic membranes of chicken embryos, as set forth below:

(a) *Virus from calves*—(1) *Quarantine.* Only calves which, prior to being placed in quarantine have reacted negatively to tuberculin, were afebrile and free of ectoparasites, and which shall have met all other applicable quarantine requirements of § 600.11(f)(2)(i) of this chapter, shall be used for vaccinia virus production. The quarantine period shall be at least 14 days. During the last 7 days of the quarantine period daily morning and afternoon rectal temperatures shall be taken and calves that do not remain afebrile during that period shall not be used for virus production.

(2) *Inoculation.* A larger area of the calf than will be used for production purposes shall be prepared in a manner comparable to that appropriate for aseptic surgery, except that the area to be inoculated must be washed free of all antiseptics that may have a deleterious effect on virus propagation. The instrument and method used for scarification must produce a uniform penetration into the epidermis but must not extend through into the corium.

(3) *Incubation.* The inoculated calf shall remain in the incubation room confined to its stall and daily morning and afternoon rectal temperatures shall be taken to determine that only the expected febrile condition occurs. If any signs of disease other than vesiculation at the inoculation site occur, the virus from that calf shall not be used for vaccine manufacture.

(4) *Harvesting.* Before harvesting, the calf shall be anesthetized and killed by exsanguination. Prior to harvesting, the inoculated area shall be thoroughly cleansed by aseptic techniques. Only the vesicular material shall be harvested.

(5) *Necropsy.* A necropsy shall be made of each production calf. The harvested material shall not be used from any animal suspected of having an infection other than vaccinia.

(b) *Virus from embryonated chicken eggs*—(1) *Eggs for production.* Embryonated chicken eggs used for propagation of vaccinia virus shall be derived from flocks found to be free of, and continuously monitored for freedom from *Salmonella pullorum,* Mycoplasma species, avian tuberculosis, fowl pox, Newcastle disease virus, Rous sarcoma virus, avian leucosis complex of viruses, and other agents pathogenic for chickens, or appropriate tests shall be performed to demonstrate freedom of the vaccine from such agents.

(2) *Harvesting.* Aseptic techniques shall be used in harvesting the chorioallantoic membranes exhibiting vesicles characteristic of vaccinia infection.

§ 630.72 Reference vaccine.

Reference Smallpox Vaccine and reconstitution fluid shall be obtained from the Center for Biologics Evaluation and Research and shall be used in all tests for determining the potency of Smallpox Vaccine.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 630.73 Potency test.

Each filling of Smallpox Vaccine shall be tested for potency by the ''pock count'' method as follows:

(a) [Reserved]

(b) *Pock counting in embryonated chicken eggs*—(1) *Dilutions* shall be made starting with no less than 0.5 ml. of the test vaccine and of the reference vaccine. The same diluent shall be used for all dilutions of both vaccines. The sample of vaccine in capillary tubes shall be obtained by pooling the contents of no less than 50 capillaries into a sterile vessel.

(2) *Inoculation of embryonated chicken eggs.* One-tenth milliliter of each dilution of test vaccine shall be inoculated onto the chorioallantoic membrane of each of at least five embryonated chicken eggs. The reference vaccine shall be tested in the same manner.